The Journal of Experimental Biology 210, 1350-1361
Published by The Company of Biologists 2007
doi:10.1242/jeb.02744

Spectral properties of identified polarized-light sensitive interneurons in the brain of the desert locust *Schistocerca gregaria*

Michiyo Kinoshita[1], Keram Pfeiffer[2,*] and Uwe Homberg[2,†]

[1]*School of Advanced Sciences, The Graduate University for Advanced Studies, Shonan Village, Hayama, Kanagawa 240-1930, Japan and* [2]*Department of Biology, Animal Physiology, University of Marburg, D-35032 Marburg, Germany*

*Present address: Department of Physiology and Biophysics, Dalhousie University, Halifax, NS, B3H 4H7, Canada
†Author for correspondence (e-mail: homberg@staff.uni-marburg.de)

Accepted 6 February 2007

Summary

Many migrating animals employ a celestial compass mechanism for spatial navigation. Behavioral experiments in bees and ants have shown that sun compass navigation may rely on the spectral gradient in the sky as well as on the pattern of sky polarization. While polarized-light sensitive interneurons (POL neurons) have been identified in the brain of several insect species, there are at present no data on the neural basis of coding the spectral gradient of the sky. In the present study we have analyzed the chromatic properties of two identified POL neurons in the brain of the desert locust. Both neurons, termed TuTu1 and LoTu1, arborize in the anterior optic tubercle and respond to unpolarized light as well as to polarized light. We show here that the polarized-light response of both types of neuron relies on blue-sensitive photoreceptors. Responses to unpolarized light depended on stimulus position and wavelength. Dorsal unpolarized blue light inhibited the neurons, while stimulation from the ipsilateral side resulted in opponent responses to UV light and green light. While LoTu1 was inhibited by UV light and was excited by green light, one subtype of TuTu1 was excited by UV and inhibited by green light. In LoTu1 the sensitivity to polarized light was at least 2 log units higher than the response to unpolarized light stimuli. Taken together, the spatial and chromatic properties of the neurons may be suited to signal azimuthal directions based on a combination of the spectral gradient and the polarization pattern of the sky.

Key words: skylight navigation, polarization vision, insect brain, color vision, spectral opponency, *Schistocerca gregaria*.

Introduction

For long-distance navigation many animals rely on external compass cues, such as the position of the sun, the moon, or the earth's magnetic field to maintain navigational directions (Rossel and Wehner, 1984; Rossel and Wehner, 1986; Lohmann and Lohmann, 1996; Wiltschko and Wiltschko, 1996; Wehner, 1997; Wehner, 2003; Dacke et al., 2003a; Dacke et al., 2003b; Mouritsen and Ritz, 2005). Sun compass navigation is a particularly common strategy. In sun compass navigation, the animals adjust their navigational direction at a certain angle to the solar azimuth, the horizontal component of the sun's position in the sky. Celestial cues other than direct sunlight, however, are also useful as a reference to the sun, especially when the sun is not visible at dawn or dusk or when it is hidden behind clouds or large objects. Scattering of sunlight in the atmosphere results in a polarization pattern, in a spectral gradient, and in an intensity gradient along the sunlit sky. Behavioral experiments have demonstrated that desert ants, monarch butterflies, dung beetles and honeybees use the celestial polarization pattern as a cue for navigation (Rossel and Wehner, 1986; Dacke et al., 2003a; Dacke et al., 2003b; Wehner, 2003; Saumann et al., 2005) (but see Stalleicken et al., 2005). Desert ants and bees, in addition, can also navigate based on the spectral gradient in the sky (Rossel and Wehner, 1984; Wehner, 1997).

The neuronal basis of polarized-light vision has been studied in several insect species. Polarized light is perceived by a small dorsal rim area (DRA) in the compound eye. Photoreceptors of the DRA show striking adaptations for detection of polarized light: they are homochromatic, have microvilli that are highly aligned in parallel, and often have wide receptive fields (Labhart and Meyer, 1999; Dacke et al., 2002; Homberg and Paech, 2002). As a result, photoreceptors in the DRA show high polarization sensitivity (Labhart and Meyer, 1999; Dacke et al., 2002; Stalleicken et al., 2006). Their axons project to dorsal areas in the lamina and medulla (Blum and Labhart, 2000; Homberg and Paech, 2002) and provide input to polarized-light sensitive interneurons (POL neurons). POL neurons show sinusoidal modulation of spiking activity depending on the e-vector angle of polarized light. Various

types of POL neuron have been characterized in the optic lobe of several species (Labhart, 1988; Homberg and Würden, 1997; Labhart, 2000; Labhart et al., 2001; Loesel and Homberg, 2001; Pfeiffer et al., 2005). Among these, POL-1 neurons of crickets with ramifications in the medulla have been studied particularly well (Labhart et al., 2001; Wehner and Labhart, 2006). Both in the field cricket and in the desert locust, POL neurons have also been reported in the central complex (Vitzthum et al., 2002; Sakura and Labhart, 2005), a brain area involved in visual memory and spatial orientation (Strauss, 2002; Liu et al., 2006). Central-complex neurons have receptive fields oriented toward the zenith and display a wide range of e-vector tunings (Homberg, 2004; Sakura and Labhart, 2005). In the desert locust *Schistocerca gregaria* these neurons are sensitive not only to polarized light but also to unpolarized light (Vitzthum et al., 2002). In contrast, the POL-1 neurons in crickets are not sensitive to unpolarized light (Labhart, 1988). This difference suggests that the sky navigation system of the desert locust might be different in certain respects from that of the field cricket.

In desert locusts, the anterior optic tubercle (AOTu) is a relay station in the polarization vision pathway from the compound eye to the central complex (Homberg et al., 2003). It receives input from line tangential neurons of the medulla. These neurons have dendritic ramifications in the dorsal rim area of the medulla and axonal projections through the anterior optic tract to the AOTu (Homberg et al., 2003). Recently, four types of POL neuron were identified in the AOTu of the locust (Pfeiffer et al., 2005). Two of them, LoTu1 (Fig. 1A) and TuTu1, innervate the lower units of the AOTu bilaterally. Both cell types respond to polarized light and to unpolarized light like POL neurons of the central complex (Fig. 1B,C). LoTu1 and TuTu1 show distinct e-vector tuning and receive polarized-light input exclusively (LoTu1) or largely (TuTu1) *via* the ipsilateral eye. Their responses to unpolarized light depend on stimulus position (Fig. 1C). Two other types of POL neuron in the AOTu have been studied less well. These neurons (TuLAL1a and TuLAL1b) project to the lateral accessory lobe and provide input to POL neurons of the central complex.

The spectral sensitivity of POL neurons in the locust is not

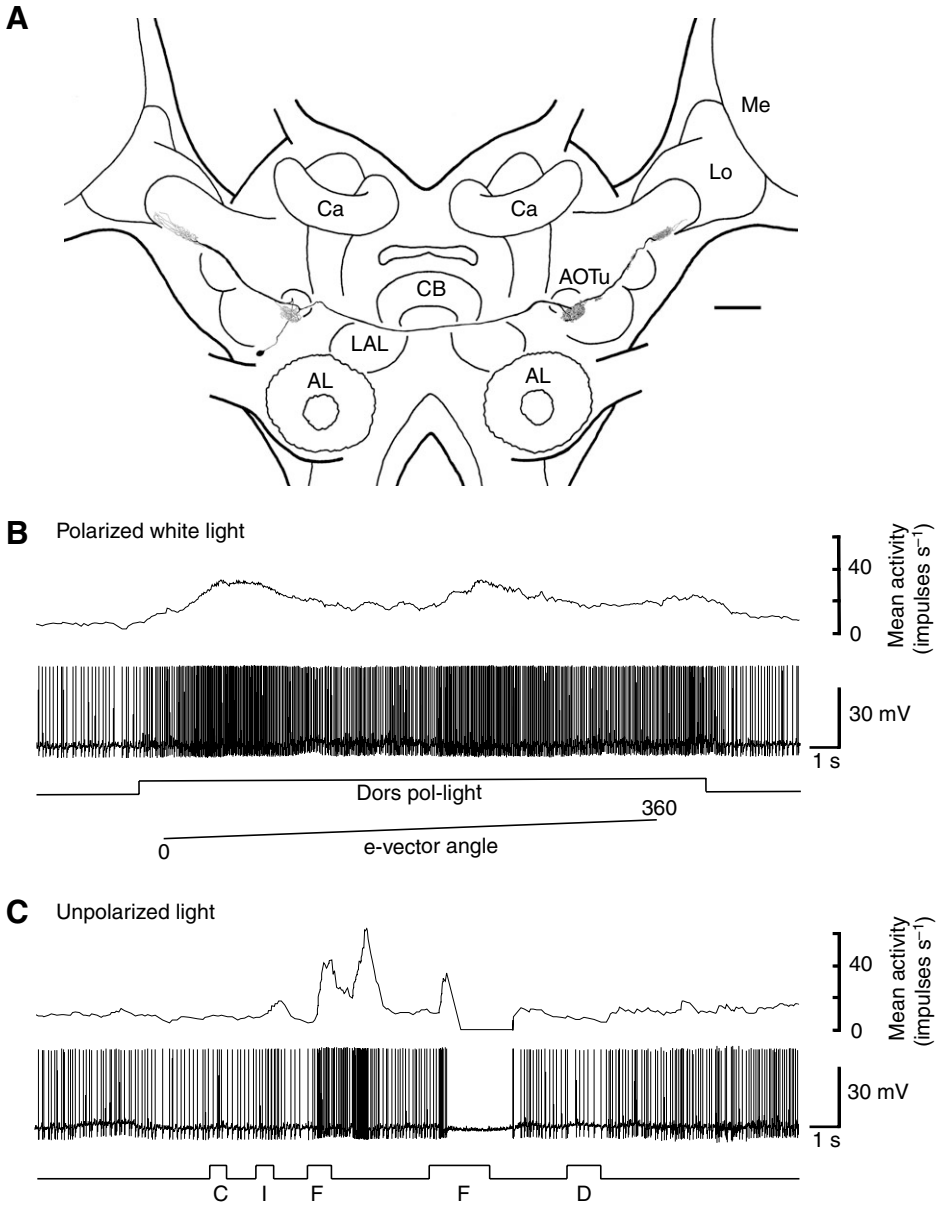

Fig. 1. Morphology and physiology of a LoTu1 neuron. (A) Schematic representation of the locust brain with arborization pattern of the polarized-light sensitive LoTu1 neuron. AL, antennal lobe; AOTu, anterior optic tubercle; Ca, calyces of mushroom body; CB, central body; LAL, lateral accessory lobe; Lo, lobula; Me, medulla. Scale bar: 200 μm. (B) Top trace shows mean spiking activity (moving average, bin width 0.5 s), middle trace shows intracellular recording of a LoTu1 neuron during zenithal stimulation with polarized light (Dors pol-light). Rotation of the polarizer through 360° (bottom trace) leads to e-vector-dependent changes in spiking activity. (C) Mean spiking activity and intracellular recording of responses to unpolarized white light applied from contralateral (C), ipsilateral (I), frontal (F) and dorsal (D) directions (150 W halogen bulb, spectral range: ~400–800 nm; visual angle: 3°; frontal: 13 μW cm^{-2}; contralateral, ipsilateral, dorsal: 2–4 μW cm^{-2}). Positive deflections in the bottom trace indicate duration of light stimuli.

known. In the DRA of *S. gregaria*, there are blue receptors peaking at 450 nm with high sensitivity to polarized light (polarization sensitivity, PS=6.92) and UV receptors peaking at 320 nm with low sensitivity to polarized light (PS=2.04) (Eggers and Gewecke, 1993). In the rest of compound eye, there are no published data from *S. gregaria*, but three types of spectral receptors were identified in *Locusta migratoria* (Vishnevskaya and Shura-Bura, 1990). These types of photoreceptor are maximally sensitive at 360 nm (UV receptor), 430 nm (blue receptor), and 530 nm (green receptor). Which type of spectral receptor dominates the responses of POL neurons in the desert locust? POL neurons in crickets and desert ants are monochromatic and tuned to the spectral sensitivity of polarized-light sensitive photoreceptors in the DRA (Labhart, 1988; Labhart, 2000). Is this also true for the locust?

In the present study, we focus on the two previously characterized bilateral POL neurons of the AOTu, LoTu1 and TuTu1. We penetrated these neurons and analyzed their spectral responses to polarized light and to unpolarized light by using a set of monochromatic filters. We show that both types of neuron are sensitive to polarized blue light. In response to unpolarized light, the spectral responses depend on stimulus position and show antagonism in the response to UV and green light. These responses might be an adaptation to the spectral gradient in the sky.

Materials and methods

Animals

We used mature female desert locusts *Schistocerca gregaria* Forskål within 1–3 weeks after imaginal moult. Locusts were reared in crowded colonies at 28°C under a light regime of 12 h:12 h light:dark at the University of Marburg. They were fed with fresh wheat leaves and wheat flakes.

Stimuli

Polarized and unpolarized monochromatic light were used for stimulation. Both types of light stimuli were provided by passing the light of a 75 W xenon lamp through a set of narrow band interference filters, neutral density filters, and a circular neutral density wedge spanning 5 log units of intensity. The interference filters and a shutter were controlled by a shutter controller (Lambda 10-2, Sutter Instruments, Novato, CA, USA). The neutral density wedge was adjusted by a custom-built control unit. Both devices were driven by a custom-built program.

Unpolarized monochromatic light was produced by passing light through one of nine interference filters with a spectral range between 330 and 600 nm (330FS10–600FS10; LOT Oriel, Darmstadt, Germany). The maximum intensity of monochromatic light was adjusted to equal photon flux at either 16.5×10^{12} or 10.6×10^{12} photons cm^{-2} s^{-1}. The intensity of the unpolarized monochromatic light was changed within a range of 3 log units with neutral density filters. Calibration of intensities was carried out with a radiometer (P-9201, Gigahertz-Optik, Puchheim, Germany). The duration of monochromatic light stimuli was either 500 ms or 1 s, separated by 1–3 s of darkness. Light passed through a UV-transmitting quartz light guide attached to a perimeter and was seen by the locust at a distance of 10 cm from the locust's head. The angular extent of the stimuli at the locust's eye was 2°. By moving the light guide along the perimeter, the light stimuli were administered from four directions: from dorsal (zenith), from frontal (elevation about 50°), and from lateral to the right or left eye at an elevation of 30–45°.

Polarized light was produced by inserting a UV transmitting polarizer (HNP'B, Polaroid, Cambridge, MA, USA) between the light guide and the animal. During stimulation, the polarizer was rotated through 360° in either direction at 20 or 21.8° s^{-1}. We stimulated with both 'white' and monochromatic polarized lights (UV, 330 nm; blue, 450 nm; green, 530 nm). The maximum intensity of each polarized monochromatic light stimulus at the surface of the locust's eye was adjusted to either 9.0×10^{12} or 10.6×10^{12} photons cm^{-2} s^{-1}. The intensity of each polarized monochromatic light was changed within a range of 4 log units with neutral density filters.

Electrophysiology

After cropping legs and abdomen, locusts were fixed to a metal holder with a wax-rosin mixture. The head capsule was opened frontally to expose the brain. The metal holder was mounted in the center of a Faraday cage. Sharp glass microelectrodes filled with 1 mol l^{-1} KCl (resistance about 50–150 MΩ) and 4% Neurobiotin in 1 mol l^{-1} KCl (Vector Laboratories, Burlingame, CA, USA) at the tip were inserted in the vicinity of the AOTu. After successful impalement we first stimulated with polarized light. If the cell was polarization-sensitive, we measured the spectral response properties to polarized light and to unpolarized light. Action potentials were amplified with a custom-made amplifier, monitored with an oscilloscope (Hameg HM 205–2; Hameg, Frankfurt/Main, Germany), digitized at 25 kHz with a Digidata 1322A (Molecular Devices, Sunnyvale, CA, USA) and stored on a personal computer using Clampex 9.2 (Molecular Devices). After recording, the neurons were injected with Neurobiotin by administering positive currents of 1–3 nA for 5–60 s.

Data analysis

The frequency of action potentials in the recordings was evaluated with the threshold detection algorithm of Clampfit 9.2 (Molecular Devices). The mean spike frequency during an interval of 1 s or 500 ms before the onset of the light stimuli was used as background activity. Responses to polarized light were analyzed using a procedure described previously (Pfeiffer et al., 2005). e-vector response plots were obtained by plotting means of spike frequencies during consecutive 10° bins of the rotating polarizer against the bin centers. e-vector angles eliciting maximal (Φ_{max}) and minimal (Φ_{min}) spike activity were determined by fitting $\sin^2$ functions to the data sets using the nonlinear least-squares Levenberg–Marquardt algorithm (Origin 6.0, Microcal, Northhampton, CA, USA). To quantify

the neuronal responses to polarized light stimuli, we calculated the response value *R*, introduced by Labhart (Labhart, 1996). Action potentials were detected by a threshold function and their number was counted in 18 consecutive bins of 20° using a custom-written semiautomatic spike2 script (Cambridge Electronic Design, Cambridge, UK). *R* was then calculated as:

$$R = \sum_{i=1}^{i=18} |n_i - \bar{n}| , \quad (1)$$

where n_i is the number of spikes in bin i and $\bar{n}$ the mean number of spikes per bin during the 360° rotation. To compare the responses to three monochromatic polarized lights, spiking activities at Φ_{max} were normalized against the maximum response. In the intensity/response plots, spiking activities at Φ_{max} were, likewise, normalized against the maximum response. Intensity/response curves were obtained by fitting the data with modified Naka–Rushton functions, $rA/rA_{max}=I^n/(I^n+K^n)$, and $rR/rR_{max}=I^n/(I^n+K^n)$, where I is stimulus intensity, rA is relative spiking activity, rR is relative response, K is stimulus intensity eliciting 50% rA_{max}, resp. rR_{max}, and n is exponent (KaleidaGraph 4.0; HULINKS, Tokyo, Japan). To compare the intensity/response curves for unpolarized and polarized light, differences between spiking activity and background activity were used as data fit to the Naka–Rushton function.

The responses to unpolarized monochromatic light were evaluated by measuring the mean spiking rate during 1 s time intervals before the onset of the stimulus, during the stimulus and after the stimulus, using a semiautomatic script. Two-sided student's *t*-tests were used to determine statistical differences in the responses to different colors.

Histology

After the Neurobiotin injection, the locust was kept at room temperature for at least 20 min to allow for diffusion of the tracer. The brain was dissected out of the head capsule and fixed overnight at 4°C in 4% paraformaldehyde, 0.25% glutaraldehyde, and 0.25% saturated picric acid in 0.1 mol l^{-1} phosphate buffer pH 7.4 (PB). Brains were subsequently embedded in gelatin/albumin and fixed overnight in 8% formaldehyde in PB at 4°C. Sections of 35 μm were cut with a vibrating blade microtome (VT–1000S, Leica, Wetzlar, Germany). They were incubated for at least 18 h at room temperature in streptavidin conjugated to horseradish-peroxidase (Amersham Buchler, Brunswick, Germany) at a dilution of 1:200 in phosphate-buffered saline containing 0.5% Triton X-100. Sections were stained with 3,3′-diaminobenzidine tetrahydrochloride and nickel ammonium sulfate as described elsewhere (Vitzthum et al., 2002). Finally, the sections were mounted on glass microslides, dehydrated, cleared in xylene, and embedded in Entellan (Merck, Darmstadt, Germany) under glass coverslips. Neurons were reconstructed using a compound microscope with camera lucida attachment. The terms ipsilateral and contralateral refer to the position of the cell body.

Results

We recorded from 32 polarization-sensitive interneurons with arborizations in the AOTu. Of these, 22 stable recordings were selected for analysis. All recordings could be classified as being from either LoTu1 (14 recordings) or TuTu1 (8 recordings) types of heterolateral interneurons of the AOTu described previously (Pfeiffer et al., 2005). The perikarya of both cell types lie in the inferior lateral protocerebrum.

LoTu1

Recordings from LoTu1 confirmed our previous findings on responses to polarized and unpolarized white light (Fig. 1) (Pfeiffer et al., 2005). Dorsal polarized light led to tonic excitation that was sinusoidally modulated in strength by the rotating e-vector (Fig. 1B). Unpolarized ipsilateral light led to an increase in spiking activity, while zenithal dorsal stimulation led to tonic inhibition. Strong frontal illumination caused an excitatory response or a phasic excitation followed by strong tonic inhibition (Fig. 1C).

In the present study we analyzed the responses of LoTu1 to monochromatic light stimuli. Dorsal polarized monochromatic light stimuli (UV, 330 nm; blue, 450 nm; green, 530 nm) at 9.0×10^9 photons cm^{-2} s^{-1} were tested in seven recordings from LoTu1. The background activity of these neurons was relatively low at 4.82±1.09 impulses s^{-1} (mean ± s.e.m.). The excitatory response of LoTu1 to polarized blue light was stronger than the response to polarized UV and green light (Fig. 2A,B). In four recordings, green light elicited virtually no response (Fig. 2A), but in three other LoTu1 cells, the response to polarized UV light was smaller than the response to polarized green light. The mean spiking activity at Φ_{max} during stimulation with polarized blue light was about two times the activity at Φ_{max} when stimulating with UV light or green light (Fig. 2B). The mean spiking activity at Φ_{max} in response to polarized UV light did not differ significantly from the response to polarized green light. The response strength *R* (for definition of *R*, see Materials and methods) showed the same results (Fig. 2B). Fig. 2C,D shows the intensity/response curves from six LoTu1s (mean background activity, 8.65±1.82 impulses s^{-1}). At the lowest light intensity of log*I*=–4(10.6×10^8 photons cm^{-2} s^{-1}) LoTu1 did not show clear responses. The activity at Φ_{max} saturated at intensities between log*I*=–2 and –1.5, the response value *R,* in contrast, only near log*I*=0. In one LoTu1, the response value *R* at maximum intensity was smaller than the response strength at log*I*=–3 and –2.

The responses of LoTu1 to unpolarized monochromatic lights (UV, 350 nm; blue, 430 nm; green, 530 nm) were different depending on eye region and wavelength (Fig. 3). The responses to unpolarized light at the lowest light intensity (log*I*=–3) were quite small. Clear responses to unpolarized light stimuli were observed at intensities above log*I*=–2. LoTu1 was inhibited by blue light from the dorsal and from the contralateral side (Fig. 3A, arrows). At intensities of log*I*=–1 and log*I*=0, LoTu1 was inhibited by both UV and blue light. These inhibitions were followed by post-inhibitory rebound

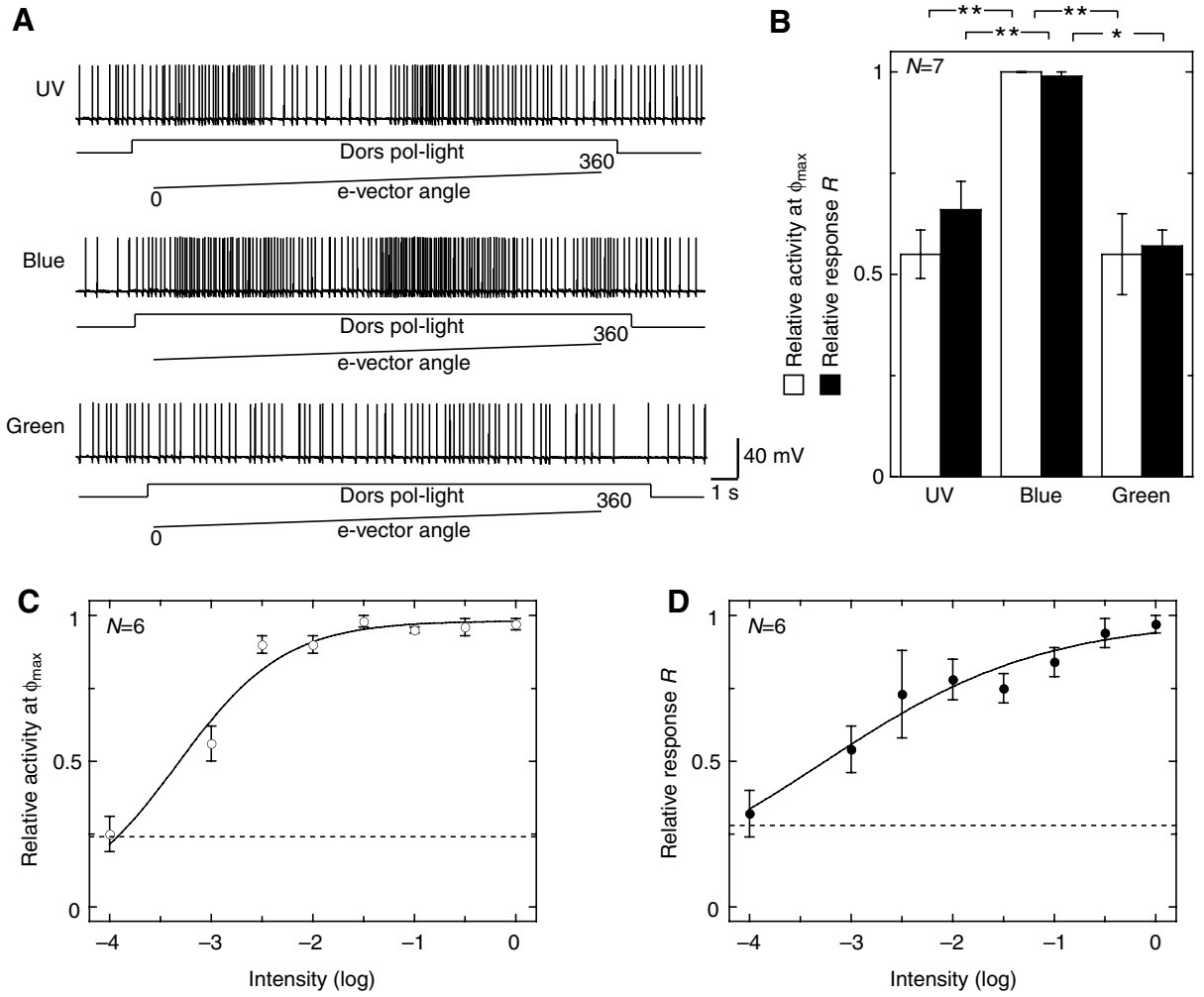

Fig. 2. Responses of LoTu1 to dorsally presented polarized light (Dors pol-light). (A) Intracellular recording traces during stimulation with three polarized monochromatic light stimuli at 9.0×10^9 photons cm^{-2} s^{-1}. Bars below the recording traces indicate the duration of the stimuli and the direction of rotation. (B) Relative spiking activities at Φ_{max} (open bars) and response strength R (solid black bars; for definition, see Materials and methods) in response to three polarized monochromatic lights (means ± s.e.m.). Relative spiking activity at Φ_{max} and R are significantly larger in response to polarized blue light than to UV and green light, $^*P<0.05$; $^{**}P<0.01$ (t-test). (C,D) Intensity/response curves of neural activity at Φ_{max} (C) and R (D) in response to polarized blue (450 nm) light (means ± s.e.m.). Maximum light intensity ($\log I=0$) is 10.6×10^{12} photons cm^{-2} s^{-1}. Solid lines show fits of Naka–Rushton functions with the fitting parameters $rA_{max}=0.98$, $K=-3.33$ log units, n=0.83 (C) and $rR_{max}=0.99$, $K=-3.28$ log units, n=0.4 (D). Broken lines indicate background activity (C) and no-stimulus R value (D).

excitations after the offset of the stimulus (Fig. 3A, open arrowheads). The strength of these rebound excitations depended on the light intensity of the stimulus (Fig. 3A). The inhibitory responses to dorsal light stimulation were stronger than those to contralateral stimulation. When light stimuli were applied ipsilaterally, LoTu1 was inhibited by UV light (Fig. 3A, arrowhead, Fig. 4B) but was excited by green light (Fig. 3A, double arrowhead, Fig. 4B). At $\log I=0$, however, one of the neurons was excited by ipsilateral UV and green light (Fig. 3A). This reversal in response to UV was possibly caused by masking of UV inhibition by a strong excitatory response to green.

The responses of LoTu1 to unpolarized light were consistent among four to six LoTu1s (Fig. 3B). When stimulated dorsally, all LoTu1s were inhibited by blue light. In response to ipsilateral stimulation, four LoTu1s were excited by green and were inhibited by UV light as shown in Fig. 3A. One of two other recordings from LoTu1s with no background activity showed an excitatory response to green light and no response to UV light. The other LoTu1 showed weak excitatory responses to UV light and green light. When light stimuli were presented from the contralateral side, three LoTu1s were inhibited by blue light, but the mean spiking activity during stimulation was not significantly lower than background activity. During ipsilateral stimulation with a series of nine monochromatic lights, LoTu1 showed clear spectral opponency (Fig. 3C). Stimulations at short wavelengths, from 330 nm to 430 nm, inhibited the neuron, whereas stimulations at long wavelengths, from 500 nm to 550 nm, excited the neuron.

In one LoTu1 neuron, we successfully recorded the

responses to polarized blue light and to unpolarized lights, adjusted to the same intensities (Fig. 4). LoTu1 (background activity 5.23 impulses s^{-1}) started to be inhibited by unpolarized dorsal blue light at log*I*=–1.5 (Fig. 4B, arrow). This inhibitory response increased with increasing light intensity. The post-inhibitory rebound excitation after the stimulus also increased depending on stimulus intensity (Fig. 4B, open arrowhead). The neuron was inhibited by unpolarized ipsilateral UV light (Fig. 4B, arrowhead). In contrast to the blue-light inhibition, this inhibitory response outlasted the stimulus and led to complete inhibition by UV light above a light intensity of log*I*=–1 (Fig. 4B, double open arrowhead). The neuron only gradually recovered to background spiking after more than 3 s following stimulation at highest intensity. The response to ipsilateral green light became apparent at a light intensity of log*I*=–1.5 (Fig. 4B,

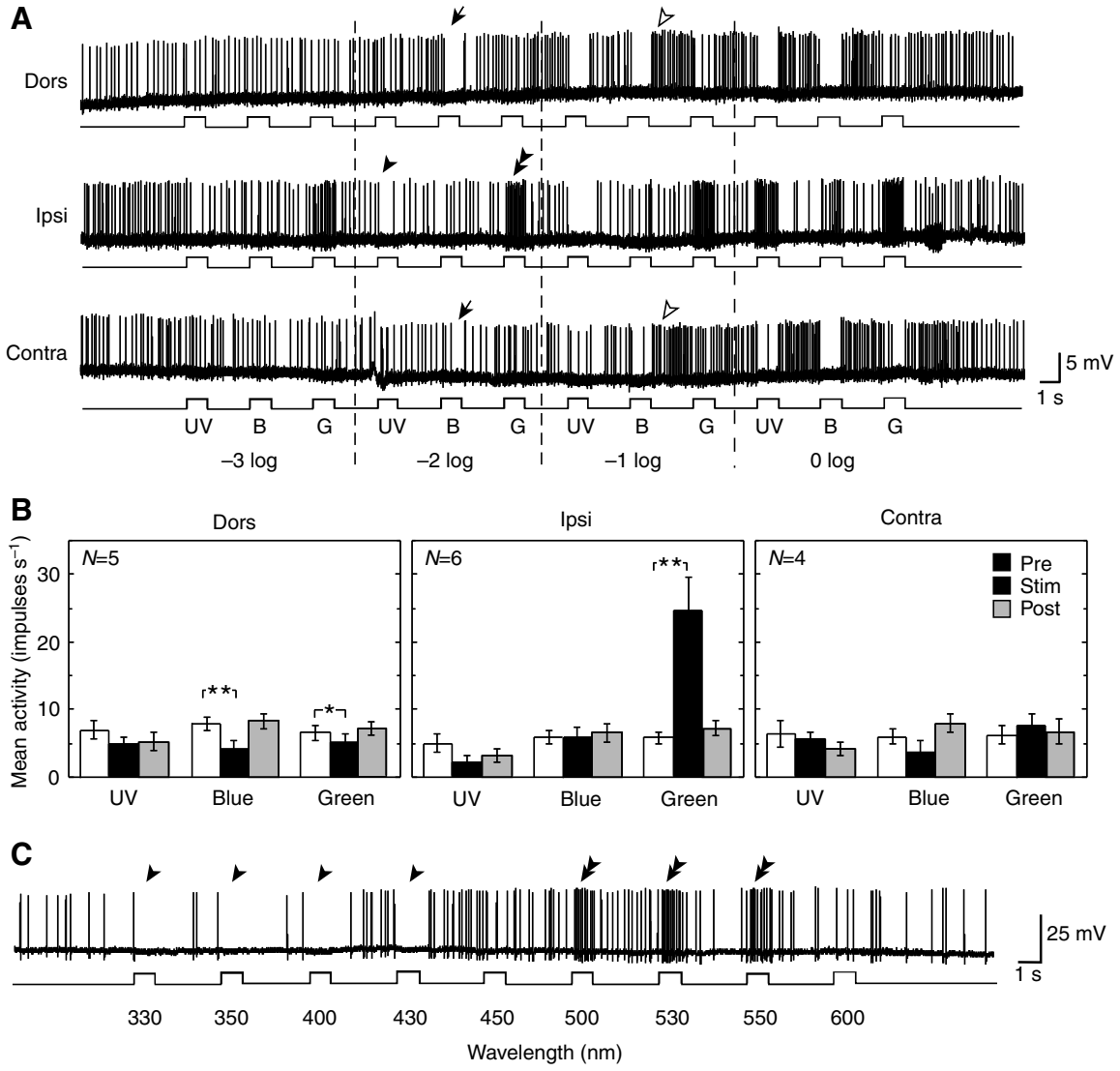

Fig. 3. Responses of LoTu1 to unpolarized monochromatic lights applied from three different directions. (A) Intracellular recording traces of responses to three unpolarized monochromatic lights (UV, 350 nm; B, 430 nm; G, 530 nm) at different intensities. Positive deflections in the traces below the spike trains indicate duration of light stimuli. Maximum intensity of each monochromatic light stimulus (0 log) was 16.5×10^{12} photons cm^{-2} s^{-1}. Stimuli were given from the zenith (Dors), ipsilateral (Ipsi, 30–45° elevation) and contralateral (Contra, 30–45° elevation) directions. Blue light from dorsal and contralateral directions leads to inhibition (arrows) followed by rebound excitation after stimulation (open arrowheads). When light stimuli were applied from the ipsilateral direction, the neuron was inhibited by UV light (arrowhead) and excited by green light (double arrowhead). (B) Responses to three unpolarized monochromatic lights at 16.5×10^{10} photons cm^{-2} s^{-1}. In each graph, activities to the three monochromatic lights are shown before stimulus onset (Pre, open bars), during stimulation (Stim, black bars), and after stimulation (Post, grey bars). LoTu1 is significantly inhibited by dorsal blue and green light and is excited by ipsilateral green light: **P*<0.05; ***P*<0.01 (*t*-test). (C) Intracellular recording showing the responses to nine unpolarized monochromatic lights from the ipsilateral direction at 16.5×10^{10} photons cm^{-2} s^{-1}. Lights at short wavelengths (330–430 nm) inhibit (arrowheads) and green lights (500–550 nm) excite the neuron (double arrowheads).

double arrowhead). Comparison of the response/intensity curves for responses to unpolarized lights and to dorsal polarized light (Fig. 4C) shows that sensitivity to polarized light is already present at intensities below log*I*=–3. In contrast, clear responses to unpolarized light only occurred above a light intensity of log*I*=–1. These results show that LoTu1 is about 2.5 log units more sensitive to polarized light than to unpolarized light. The dynamic range of intensity coding for polarized and unpolarized light was very narrow and covered only about 1–1.5 log units.

TuTu1

We recorded the responses to dorsal polarized monochromatic UV, blue and green light stimuli from three TuTu1 neurons. The mean background activity of these neurons was 21.03±6.24 impulses s^{-1}, considerably higher than the activity of LoTu1. TuTu1 neurons showed polarization-opponency in response to the rotating polarizer (see also Pfeiffer et al., 2005). This means that TuTu1 neurons were maximally excited at Φ_{max} and were maximally inhibited at an e-vector orientation orthogonal to Φ_{max} (Φ_{min}). The opponent response to polarized blue light was stronger than the responses to polarized UV and polarized green light (Fig. 5A,B). In two neurons, the response to polarized UV light was slightly stronger than that to polarized green light (Fig. 5A). The response strength *R* to polarized light was significantly higher at 450 nm (blue) than at 350 nm (UV) or at 530 nm (green) (Fig. 5B). In contrast, the neural activity at Φ_{max} was not significantly different between the responses to the three monochromatic polarized lights. Fig. 5C,D shows the intensity/response curves of three recordings from TuTu1. The neural activity at Φ_{max} and the response amplitude increased with increasing stimulus intensity. TuTu1 did not respond to polarized light at log*I*=–4. At a light intensity of log*I*=–2, both the activity at Φ_{max} and the response saturated.

We recorded the responses to unpolarized monochromatic lights from four TuTu1 neurons, but the responses were not consistent among the recordings. Fig. 6 shows two examples of the responses to unpolarized monochromatic lights applied to different eye regions. In Fig. 6A, TuTu1 showed spectral-opponency when light stimuli were applied from the ipsilateral direction. This TuTu1 was excited by UV light (Fig. 6A, double arrowhead) and was inhibited by green light (Fig. 6A, open arrowheads). When contralateral light stimuli were applied, the neuron showed an inhibitory response (Fig. 6A, arrow) with post-rebound excitation (Fig. 6A, open double arrowheads) to blue light. Another TuTu1 was inhibited by blue light at maximum intensity from dorsal and contralateral directions (Fig. 6B, arrows). This neuron was excited by both UV light and blue light coming from the ipsilateral side

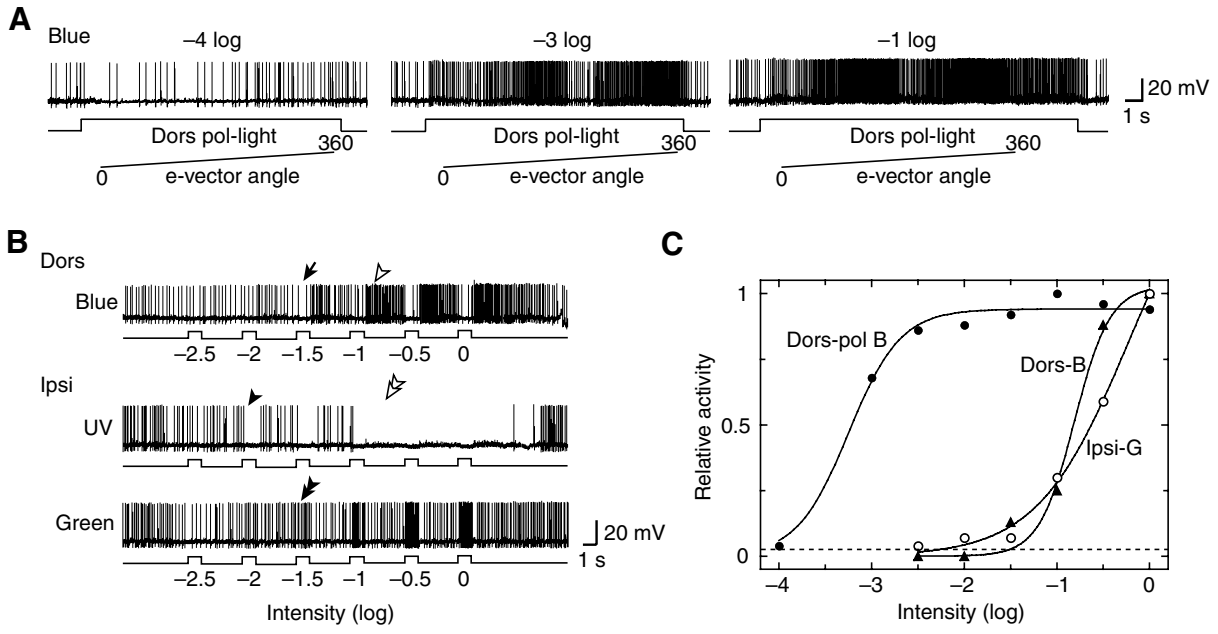

Fig. 4. Comparison of sensitivities to polarized light and unpolarized light in LoTu1. (A) Intracellular recording traces during stimulation with dorsal polarized blue (450 nm) light (Dors pol-light) at three intensities. (B) Intracellular recording of responses to unpolarized monochromatic light stimuli with increasing intensity. Inhibition to dorsal blue light becomes obvious at log*I*=–1.5 (arrow); it is followed by rebound excitation (open arrowhead). Ipsilateral UV light at log*I*=–2 inhibits the neuron (arrowhead). At log*I*=–1, the neuron is completely inhibited including the inter-stimulus intervals (double open arrowhead). Ipsilateral green light leads to excitation beginning at log*I*=–2 (double arrowhead). Maximum light intensity for all stimuli (log*I*=0) was 10.6×10^{12} photons cm^{-2} s^{-1}. (C) Intensity/response curves for dorsal polarized blue (450 nm) light at Φ_{max} (Dors-pol B), dorsal unpolarized blue light (Dors-B), and unpolarized ipsilateral green light (Ipsi-G). The inhibitory responses to Dors-B are shown as normalized values of activity during stimulation minus activity before stimulation for better comparison. Solid lines are fits of Naka–Rushton functions with the fitting parameters rA_{max}=0.94, *K*=–3.25 log units, n=1.54 (Dors-pol B), rA_{max}= 1.03, *K*=–0.81 log units, n=2.27 (Dors B), and rA_{max}=1.63, *K*=–0.23 log units, n=0.88 (Ipsi G). Broken line indicates background activity.

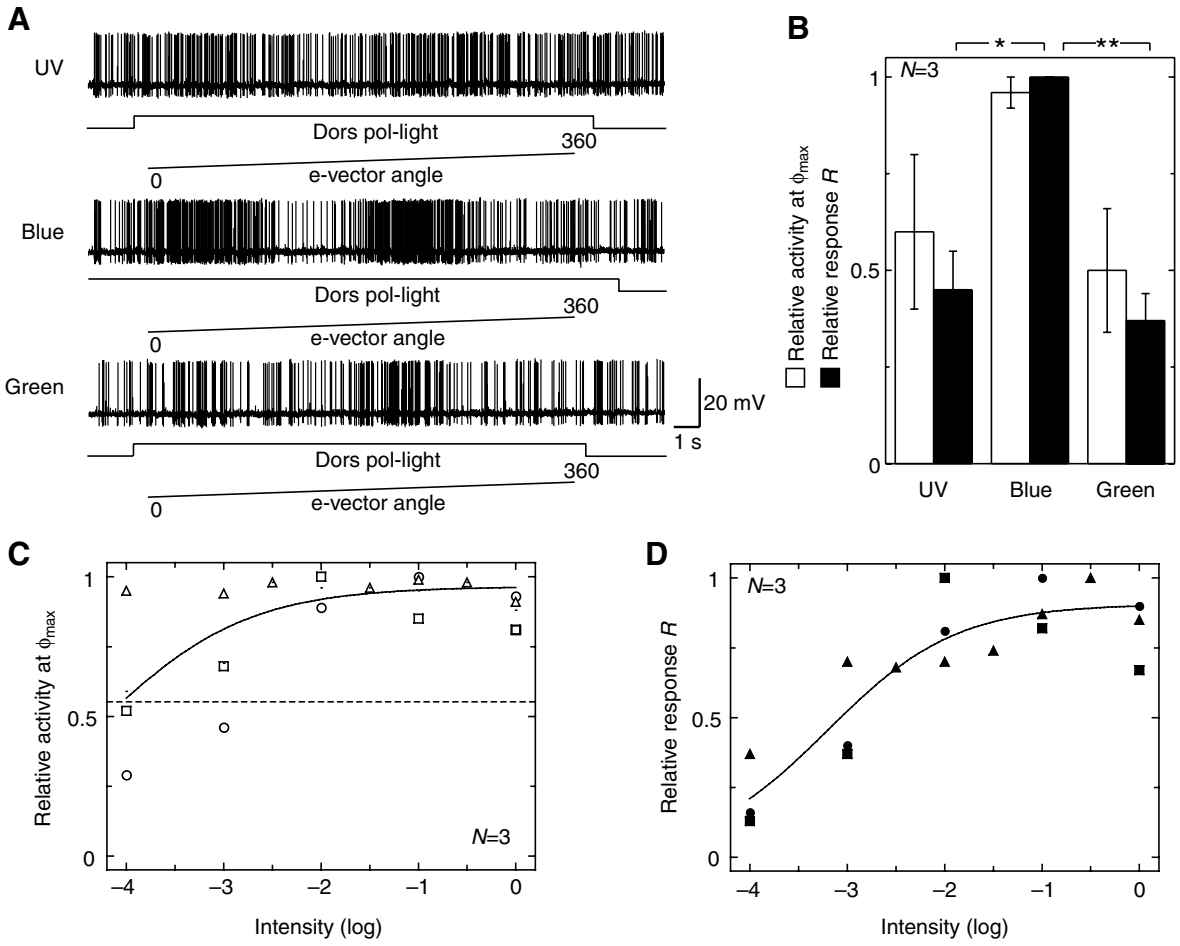

Fig. 5. Responses of TuTu1 to dorsal polarized light. (A) Intracellular recording traces of a TuTu1 during stimulation with three polarized monochromatic lights at 9.0×10^9 photons cm^{-2} s^{-1}. (B) Relative spiking activities at Φ_{max} (open bars) and response strength R (solid black bars) in response to three polarized monochromatic lights (means ± s.e.m.), $^*P<0.05$; $^{**}P<0.01$ (t-test). (C,D) Intensity/response curves of neural activity at Φ_{max} (C) and response value R (D) in response to dorsally presented polarized blue (450 nm) light. Symbol shapes represent different recordings. Maximum intensity (log$I$=0) is 10.6×10^{12} photons cm^{-2} s^{-1}. Solid lines show fits of Naka–Rushton functions with the fitting parameters rA_{max}=0.96, K=–4.26 log units, n=0.58 (C) and rR_{max}=0.90, K=–3.19 log units, n=0.66 (D). Broken line in C indicates background activity.

(Fig. 6B, arrowheads), but showed no clear response to green light. The third TuTu1 (not shown) was excited by UV light presented dorsally and ipsilaterally, but did not respond to blue light and green light from any directions. The last TuTu1 showed very weak responses that were similar in their properties to those of the neuron of Fig. 6A.

Discussion

We have analyzed the spectral and polarization properties of two identified polarization-sensitive neurons, LoTu1 and TuTu1, in the brain of the desert locust. For unbiased signalling of e-vector orientations, polarization-sensitive interneurons in other species, such as the cricket, were shown to receive exclusive input from homochromatic photoreceptors (Labhart, 1988). In contrast, both LoTu1 and TuTu1 neurons of the locust receive input from homochromatic blue polarized-light sensitive photoreceptors (Figs 2, 5, 7), as well as from polarization insensitive UV and green receptors (Figs 3, 4, 6, 7). Firm conclusions on the biological significance of these wavelength-specific responses await further studies. An attractive hypothesis, however, is that through chromatic contrast signalling spectral gradients in the sky might contribute to sky compass coding in these two neurons.

Spectral sensitivity of responses to polarized light

In the DRA of the desert locust, UV receptors with low polarization sensitivity and blue receptors with high polarization sensitivity have been detected (Eggers and Gewecke, 1993). LoTu1 and TuTu1 were most sensitive to dorsally presented polarized blue light and showed much lower sensitivity to polarized UV and green light (Fig. 2A,B, Fig. 5A,B). This result is consistent with the fact that only blue receptors in the locust DRA showed high polarization sensitivity (Eggers and Gewecke, 1993) and indicates that the polarized-light sensitivity of LoTu1 and TuTu1 neurons is

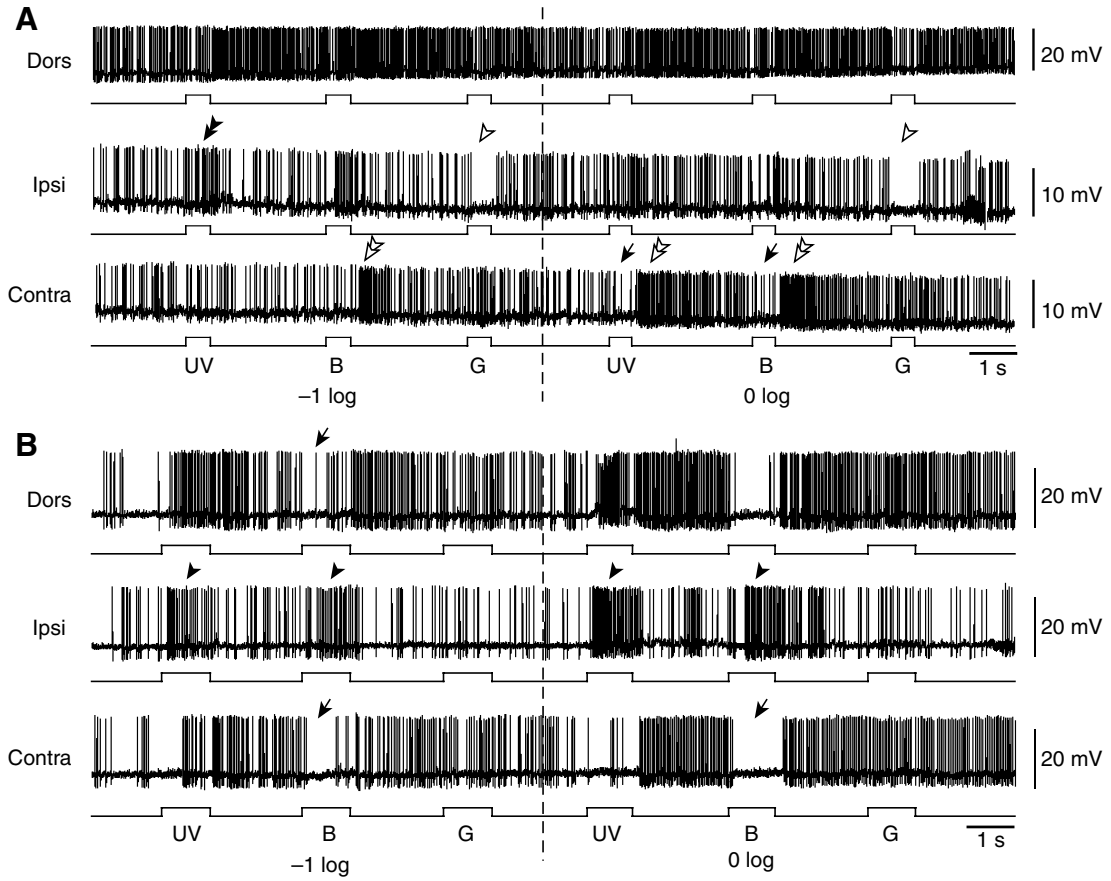

Fig. 6. Intracellular recording traces of responses to three unpolarized monochromatic light stimuli in TuTu1 neurons. (A) TuTu1 is excited by UV light (double arrowhead) and is inhibited by green light (open arrowhead) when stimuli are applied from the ipsilateral direction (Ipsi). The neuron shows inhibitory responses (arrows) with rebound excitation (double open arrowheads) to UV and blue light applied from the contralateral direction (Contra). (B) In another recording TuTu1 is inhibited by dorsal (Dors) and contralateral blue light (arrows). Ipsilateral UV and blue light lead to excitations (arrowheads). Positive deflections in the bottom trace indicate duration of light stimuli. Maximum intensity (0 log) is 16.5×10^{12} photons cm^{-2} s^{-1}.

based on blue receptors in the DRA. The polarization vision system of crickets, likewise, depends on blue photoreceptors, demonstrated behaviorally, in photoreceptor recordings, and in intracellular recordings from POL interneurons (Labhart, 1988) (reviewed by Labhart and Petzold, 1993). In contrast, polarization vision in hymenopteran species (honeybee, desert ant) relies on UV light (reviewed by Wehner and Labhart, 2006).

The absolute sensitivity for polarized light is similar in LoTu1 and TuTu1. The response threshold for polarized light was at a light intensity of log$I$=–3.5 to –3, and saturation of the response at Φ_{max} was reached at an intensity of log$I$=–2 (Fig. 2C, Fig. 5C). This means that 1–1.5 log units above threshold, the responses of both POL neurons at Φ_{max} are intensity independent. While the response strength R of TuTu1 showed a similar intensity dependence (Fig. 5D), the R value of LoTu1 increased over 3–4 log units of light intensity and only reached saturation around log$I$=0 (Fig. 2D). A likely reason for this may be the increasing contribution of a polarization-insensitive inhibition of LoTu1 by dorsal blue light above log$I$=–2, as shown in Fig. 4C. The cricket POL-1 neuron, in contrast, shows maximum response within 1 log unit of light intensity (Labhart, 1988; Labhart et al., 2001). Above a light level of about 3×10^8 photons cm^{-2} s^{-1} of blue light (443 nm), its e-vector response becomes intensity independent by receiving antagonistic input from photoreceptors with mutually orthogonal microvilli orientation. The POL-1 neuron is, therefore, at least 2 log units more sensitive to polarized light than the two locust neurons studied here.

Spectral sensitivity of responses to unpolarized light

The spectral responses of the bilateral POL neurons to unpolarized light are surprisingly complex. All three types of spectral receptors in the compound eye contribute to the unpolarized light response. The spectral responses are different at different stimulus positions, indicating that the set of spectral inputs contributing to the unpolarized light responses differ considerably depending on the eye region (Fig. 7).

LoTu1 and one subtype of TuTu1 receive inhibitory input from blue receptors in dorsal eye regions (Fig. 3, Fig. 6). The sensitivity to dorsally presented blue light may code for brightness of the blue sky. At very high light intensities, the

inhibition in LoTu1 by blue light can strongly suppress the responses to polarized light and may even become apparent when using polarized light as the stimulus.

In addition, the spectral response to unpolarized light shows opponency in the response to UV light and green light in both bilateral POL neurons when light was presented from the ipsilateral side (Fig. 3, Fig. 6). Spectral opponency is a widespread phenomenon in color vision and has been demonstrated in neurons of the optic lobe of the honeybee (Kien and Menzel, 1977) and migratory locust (Osorio, 1986). Sustaining responses and narrow receptive fields of some green-UV color opponent neurons of the locust medulla suggested that they might play a role in maintaining flight posture relative to the horizon (Osorio, 1986). Likewise, the spectral opponency in LoTu1 and TuTu1 may not contribute to true color vision, but may rather serve to evaluate the spectral gradient in the sky. At positions near the sun, the chromatic contrast between long (green) and short (UV) wavelength light is high, but becomes smaller with increasing angular distance to the sun in the anti-solar hemisphere (Rossel and Wehner, 1984; Coemans et al., 1994). To process all spectral information in the sky, an animal may code the intensity of blue light as a reference and, at the same time, the difference of intensities between green light and UV light. To further substantiate the hypothesis that LoTu1 and TuTu1 neurons integrate polarization and chromatic contrast of the sky, it will be necessary, however, to examine the azimuthal dependence and receptive fields of the chromatic responses in more detail and to test the combined effects of polarized and chromatic stimuli on the responses of the neurons.

Pathway of visual inputs to LoTu1 and TuTu1

Which neurons provide input to the bilateral POL neurons? Previous anatomical studies have shown that medulla line tangential neurons are the most promising candidates to provide visual input from the ipsilateral eye (Homberg et al., 2003). These neurons have small diameter processes along the dorso-ventral axis of the medulla – in addition to ramifications in the dorsal rim area of the medulla – and send direct processes to the lower unit of the AOTu. This morphology is ideally suited to integrate inputs from the DRA and the main retina of the compound eye. In both LoTu1 and TuTu1, ramifications in the ipsilateral AOTu are of smooth appearance and, therefore, most likely dendritic, while arborizations in the contralateral AOTu have a beaded or varicose appearance and are, therefore, most likely axonal (Pfeiffer et al., 2005). These morphologies suggest that the bilateral POL neurons receive information only from the ipsilateral eye. In fact, LoTu1 receives polarized-light input only through the ipsilateral eye, while polarization sensitivity in TuTu1 is dominated by ipsilateral eye input (Pfeiffer et al., 2005). In addition, our present study suggests that TuTu1 at least responds to unpolarized light perceived by both eyes. The information from the contralateral eye may in part originate from the counterpart LoTu1 and TuTu1 neurons of the other brain hemisphere. In addition, medulla line tangential neurons without ramifications in the DRA also project to the lower unit of AOTu (U.H., unpublished observation). These neurons may provide selectively unpolarized light inputs to the bilateral POL neurons in the AOTu.

Integration of unpolarized and polarized light signals in orientation

The bilateral POL neurons may be suited to integrate information on the celestial polarization pattern and on the spectral gradient in the sky. Polarized light information at the zenith is used for coding the orientation of the body axis relative to the solar meridian. However, sky polarization alone is not sufficient to signal solar azimuth unambiguously, since e-vector orientations alone do not allow the animal to discriminate whether the sun occurs at an azimuth φ or at an azimuth φ+180°. POL neurons in the locust, as reported here, might resolve this problem by coding not only the e-vector angle of polarized light but also the spectral gradient in the sky.

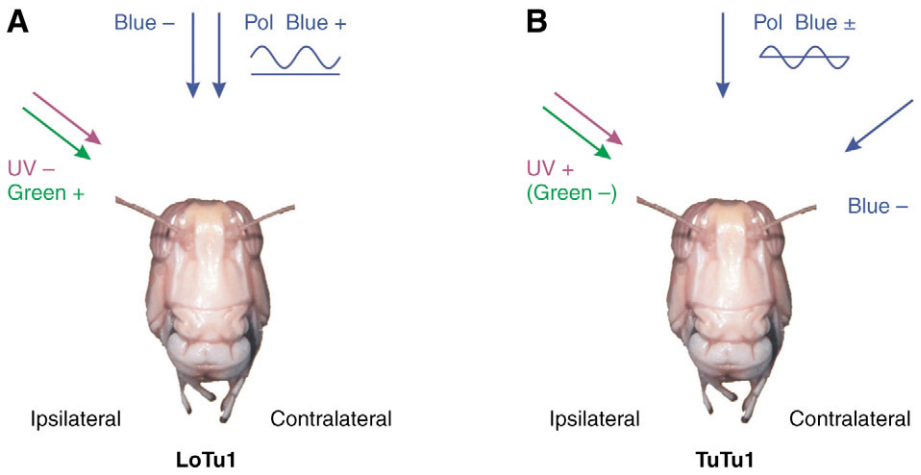

Fig. 7. Summary diagram of response properties of LoTu1 and TuTu1. (A) LoTu1 neurons are excited by ipsilateral unpolarized green light at elevations between 30 and 45° (Green +). Unpolarized UV light from the same range of directions leads to inhibition (UV –). The neurons are also inhibited by dorsal unpolarized blue light (Blue –). Dorsal polarized blue light activates LoTu1 neurons (Pol Blue +); the strength of activation is dependent on the e-vector orientation. (B) TuTu1 neurons are excited by ipsilateral unpolarized UV light at elevations between 30 and 45° (UV +). Unpolarized green light from the same directions leads to inhibition (Green –, observed in half of the recordings). Contralateral blue light at elevations between 30 and 45° (Blue –) inhibits TuTu1 neurons. Dorsal polarized blue light leads to sinusoidal modulation of the neuronal activity with excitatory and inhibitory components depending on the e-vector orientation (Pol Blue ±).

Locusts may, in addition, use intensity gradients in the sky for orientation, but whether and how intensity information is actually integrated in the compass orientation system remains to be seen. Behavioral experiments in homing bees have shown that intensity gradients do not provide substantial information for navigation (Rossel and Wehner, 1984).

The absolute sensitivity to polarized light is about 2 log units higher than the sensitivity to unpolarized light, as shown for LoTu1 (Fig. 4C). This suggests that, depending on light intensities, polarization input or unpolarized light input may dominate the responses of the neurons. At low light intensities, before dawn and after sunset or under a partly cloudy sky, the polarization vision system may be more important for orientation behavior. However, when the sky is clear and the sun is directly visible, sky chromatic contrast may prevail and provide the relevant information for orientation.

This study is the first to address the possibility that both e-vector angle of polarized light and the spectral gradient in the sky are encoded in the same neural system underlying compass orientation in an insect. Our results support behavioral data indicating that celestial compass orientation in bees and desert ants relies on both the celestial polarized-light pattern and the spectral gradient in the sky (Wehner, 1989). Observations in bees, ants, and pigeons suggest that these animals evaluate the intensity of long wavelength light with respect to the relatively isotropic UV background for sun compass navigation (Rossel and Wehner, 1984; Coemans et al., 1994; Wehner, 2003). Our findings of spectral opponency in the responses to unpolarized light fit these behavioral observations well and may be a first step in understanding how the integration of different celestial cues used for spatial orientation is organized in the brain.

List of symbols and abbreviations

A	spiking activity
AOTu	anterior optic tubercle
DRA	dorsal rim area of the compound eye
I	stimulus intensity
K	intensity eliciting 50% rA_{max}, resp. rR_{max}
LoTu1	lobula-tubercle neuron 1
n	exponent
PB	phosphate buffer
POL	polarized-light sensitive
PS	polarization sensivity
R	response value
rA	relative spiking activity
rR	relative response
TuTu1	tubercle-tubercle neuron 1
UV	ultraviolet
Φ_{max}, Φ_{min}	e-vector angle eliciting maximal/minimal spiking activity

We thank K. H. Herklotz for assistance with insect rearing, and Dr P. Galland for access to his radiometer. This work was supported by DFG grant HO 950/16-1 to U. Homberg and a fellowship by the Canon Foundation in Europe to M. Kinoshita.

References

Blum, M. and Labhart, T. (2000). Photoreceptor visual fields, ommatidial array, and receptor axon projections in the polarisation-sensitive dorsal rim area of the cricket compound eye. *J. Comp. Physiol. A* **186**, 119-128.

Coemans, M. A. J. M., Vos Hzn, J. J. and Nuboer, J. F. W. (1994). The relation between celestial colour gradients and the position of the sun, with regard to the sun compass. *Vision Res.* **34**, 1461-1470.

Dacke, M., Nordström, P., Scholtz, C. H. and Warrant, E. J. (2002). A specialized dorsal rim area for polarized light detection in the compound eye of the scarab beetle *Pachysoma striatum*. *J. Comp. Physiol. A* **188**, 211-216.

Dacke, M., Nordström, P. and Scholtz, C. (2003a). Twilight orientation to polarised light in the crepuscular dung beetle *Scarabaeus zambesianus*. *J. Exp. Biol.* **206**, 1535-1543.

Dacke, M., Nilsson, D. E., Scholtz, C. H., Byrne, M. and Warrant, E. J. (2003b). Animal behaviour: insect orientation to polarized moonlight. *Nature* **424**, 33.

Eggers, A. and Gewecke, M. (1993). The dorsal rim area of the compound eye and polarization vision in the desert locust (*Schistocerca gregaria*). In *Sensory Systems of Arthropods* (ed. K. Wiese, F. G. Gribakin, A. V. Popov and G. Renninger), pp. 101-109. Basel: Birkhäuser.

Homberg, U. (2004). In search of the sky compass in the insect brain. *Naturwissenschaften* **91**, 199-208.

Homberg, U. and Paech, A. (2002). Ultrastructure and orientation of ommatidia in the dorsal rim area of the locust compound eye. *Arthropod Struct. Dev.* **30**, 271-280.

Homberg, U. and Würden, S. (1997). Movement-sensitive, polarization-sensitive, and light-sensitive neurons of the medulla and accessory medulla of the locust, *Schistocerca gregaria*. *J. Comp. Neurol.* **386**, 329-346.

Homberg, U., Hofer, S., Pfeiffer, K. and Gebhardt, S. (2003). Organization and neural connections of the anterior optic tubercle in the brain of the locust, *Schistocerca gregaria*. *J. Comp. Neurol.* **462**, 415-430.

Kien, J. and Menzel, R. (1977). Chromatic properties of interneurons in the optic lobes of the bee. II. Narrow band and colour opponent neurons. *J. Comp. Physiol.* **113**, 35-53.

Labhart, T. (1988). Polarization-opponent interneurons in the insect visual system. *Nature* **331**, 435-437.

Labhart, T. (1996). How polarization-sensitive interneurones of crickets perform at low degrees of polarization. *J. Exp. Biol.* **199**, 1467-1475.

Labhart, T. (2000). Polarization-sensitive interneurons in the optic lobe of the desert ant *Cataglyphis bicolor*. *Naturwissenschaften* **87**, 133-136.

Labhart, T. and Meyer, E. P. (1999). Detectors for polarized skylight in insects: a survey of ommatidial specializations in the dorsal rim area of the compound eye. *Microsc. Res. Tech.* **47**, 368-379.

Labhart, T. and Petzold, J. (1993). Processing of polarized light information in the visual system of crickets. In *Sensory Systems of Arthropods* (ed. K. Wiese, F. G. Gribakin, A. V. Popov and G. Renninger), pp. 158-169. Basel: Birkhäuser.

Labhart, T., Petzold, J. and Helbling, H. (2001). Spatial integration in polarization-sensitive interneurones of crickets: a survey of evidence, mechanisms and benefits. *J. Exp. Biol.* **204**, 2423-2430.

Liu, G., Seiler, H., Wen, A., Zars, T., Ito, K., Wolf, R., Heisenberg, M. and Liu, L. (2006). Distinct memory traces for two visual features in the *Drosophila* brain. *Nature* **439**, 551-556.

Loesel, R. and Homberg, U. (2001). Anatomy and physiology of neurons with processes in the accessory medulla of the cockroach *Leucophaea maderae*. *J. Comp. Neurol.* **439**, 193-207.

Lohmann, K. and Lohmann, C. (1996). Orientation and open-sea navigation in sea turtles. *J. Exp. Biol.* **199**, 73-81.

Mouritsen, H. and Ritz, T. (2005). Magnetoreception and its use in bird navigation. *Curr. Opin. Neurobiol.* **15**, 406-414.

Osorio, D. (1986). Ultraviolet sensitivity and spectral opponency in the locust. *J. Exp. Biol.* **122**, 193-208.

Pfeiffer, K., Kinoshita, M. and Homberg, U. (2005). Polarization-sensitive and light-sensitive neurons in two parallel pathways passing through the anterior optic tubercle in the locust brain. *J. Neurophysiol.* **94**, 3903-3915.

Rossel, S. and Wehner, R. (1984). Celestial orientation in bees: the use of spectral cues. *J. Comp. Physiol. A* **155**, 605-613.

Rossel, S. and Wehner, R. (1986). Polarization vision in bees. *Nature* **323**, 128-131.

Sakura, M. and Labhart, T. (2005). Polarization-sensitive neurons in the central complex of the cricket, *Gryllus bimaculatus*. *Neuroforum* **2005**, Suppl. 154B.

Sauman, I., Briscoe, A. D., Zhu, H., Shi, D., Froy, O., Stalleicken, J., Yuan,

Q., Casselman, A. and Reppert, S. M. (2005). Connecting the navigational clock to sun compass input in monarch butterfly brain. *Neuron* **46**, 457-467.

Stalleicken, J., Mukhida, M., Labhart, T., Wehner, R., Frost, B. and Mouritsen, H. (2005). Do monarch butterflies use polarized skylight for migratory orientation? *J. Exp. Biol.* **208**, 2399-2408.

Stalleicken, J., Labhart, T. and Mouritsen, H. (2006). Physiological characterization of the compound eye in monarch butterflies with focus on the dorsal rim area. *J. Comp. Physiol. A* **192**, 321-331.

Strauss, R. (2002). The central complex and the genetic dissection of locomotor behaviour. *Curr. Opin. Neurobiol.* **12**, 633-638.

Vishnevskaya, T. M. and Shura-Bura, T. M. (1990). Spectral sensitivity of photoreceptors and spectral inputs to the neurons of the first optic ganglion in the locust (*Locusta migratoria*). In *Sensory Systems and Communication in Arthropods* (ed. F. G. Gribakin, K. Wiese and A. V. Popov), pp. 106-111. Basel: Birkhäuser.

Vitzthum, H., Müller, M. and Homberg, U. (2002). Neurons of the central complex of the locust *Schistocerca gregaria* are sensitive to polarized light. *J. Neurosci.* **22**, 1114-1125.

Wehner, R. (1989). The hymenopteran skylight compass: matched filtering and parallel coding. *J. Exp. Biol.* **146**, 63-85.

Wehner, R. (1997). The ant's celestial compass system: spectral and polarization channels. In *Orientation and Communication in Arthropods* (ed. M. Lehrer), pp. 145-185. Basel: Birkhäuser.

Wehner, R. (2003). Desert ant navigation: how miniature brains solve complex tasks. *J. Comp. Physiol. A* **189**, 579-588.

Wehner, R. and Labhart, T. (2006). Polarization vision. In *Invertebrate Vision* (ed. E. J. Warrant and D.-E. Nilsson), pp. 291-348. Cambridge: Cambridge University Press.

Wiltschko, W. and Wiltschko, R. (1996). Magnetic orientation in birds. *J. Exp. Biol.* **199**, 29-38.